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Wetting and drying effects on animal/microbial mediated nitrogen mineralization and mineral element losses from deciduous forest litter and raw humus

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With 2 figures

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1. Introduction

The process of nitrogen mineralization by microorganisms is generally considered to proceed through an initial phase in which nitrogen is immobilized followed, over a variable period of time, by net mineralization as the fungi and bacteria become carbon limited (Swift *et al.* 1979). The time course of these events is disrupted by abiotic processes (wet/dry and freeze/thaw events) and by biotic processes (notably the feeding activities of soil invertebrates). The flush of mineral nitrogen which follows the wetting of dried soil is well documented (Powlson & Jenkinson 1976) and there is increasing evidence of the importance of soil invertebrates in nitrogen mineralization processes (see reviews in Anderson *et al.* 1984, Fitter *et al.* 1985). There is, however, little information on the interactions between the biotic and abiotic processes.

Witkamp & Frank (1970) carried out laboratory studies on the effects of millipedes on mineral element mobilization (excluding nitrogen) from leaf litter under different temperature and moisture regimes. They concluded that invertebrates may be important under cool, moist conditions but at high temperatures in the field the desiccation of the litter layers inhibits animal feeding and the disruptive effects of wetting and drying events were more important in reducing microbial immobilization.

We have shown in laboratory experiments, carried out under conditions where moisture was not limiting, that the effects of millipedes on nitrogen mineralization show a marked temperature response over the range from 5 °C to 15 °C (Anderson *et al.* 1983a, 1986). We report here the effects of drying and rewetting litter and humus on these animal-mediated nitrogen and mineral element fluxes.

2. Materials and methods

These experiments were carried out using one series of experimental systems set up to quantify the effects of litter-feeding macrofauna on nitrogen and mineral element fluxes in three deciduous woodlands (Anderson *et al.* 1986). Results are presented for the Stoke Woods site (NGR 925961) which is a mixed oak/beech stand (predominantly *Quercus robur* with *Fagus sylvatica*) growing on Culm measure shale, a low base-status parent material. There is a well developed organic horizon, approximately 5 cm in depth with 80% ignition losses (400 °C) and a moder humus form. Mean monthly pH values lie in the range 3.7 to 4.5.

Fermentation layer (F) and humus layer (H) materials were collected in January 1984. The materials were air dried, lightly crushed (after removing the mid ribs from leaves) and then sieved to prepare a 5—20 mm litter fraction and a 1—3 mm fraction of humus. Mean total nitrogen (Kjeldahl) concentrations for these fractions (in mg N g^{-1} dry mass $\pm$ 1 SE) were 12.7 $\pm$ 0.3 for the F-layer and 15.3 $\pm$ 0.3 for the H-layer.

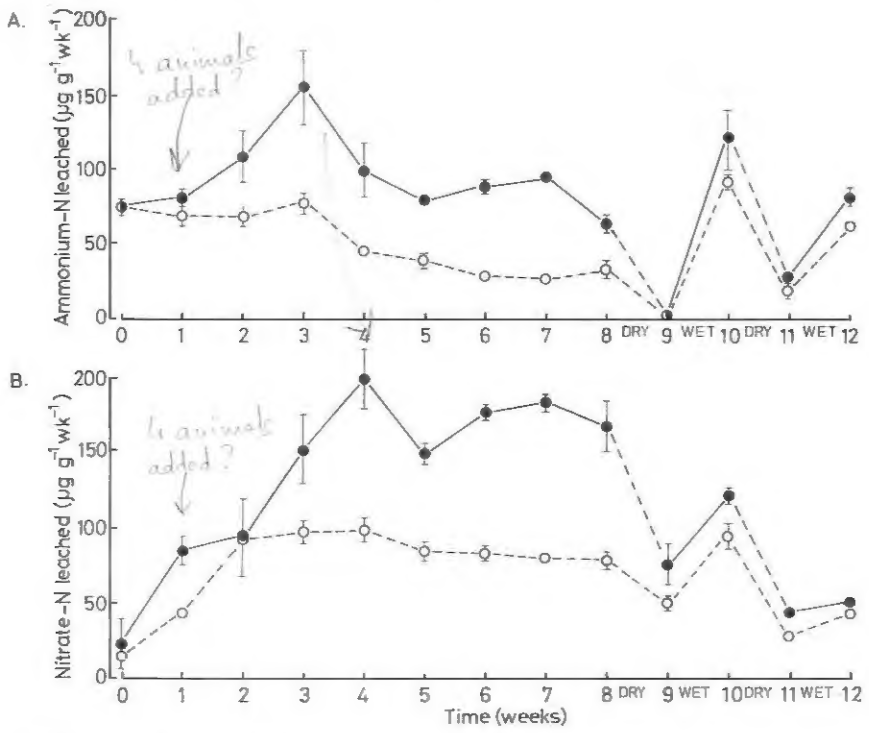
Masses of material, equivalent to oven dry masses of 2 g leaves and 6 g humus were placed in experimental chambers (Anderson & Ineson 1982), rewetted, inoculated with a suspension of fresh material, and then incubated at room temperature for 2 weeks to allow similar development of the microflora in aliquots of material. The chambers were then allocated at random to constant tem-

perature cabinets at 5 °C, 10 °C and 15 °C and 4 millipedes *(Glomeris marginata)*, with a total mass of 0.41 g, were added after 7 days at the experimental temperature. Treatments without animals were used as controls. Three replicates were made of each treatment which were arranged in a randomized block design under each temperature regime. Determinations of elements mobilized by treatments were made by irrigating the experimental material with 60 ml distilled water introduced through a tube in the base of the experimental chambers (Anderson & Ineson 1982). Leaching and leachate analyses for ammonium, nitrate, calcium, potassium and sodium was carried out every seventh day for 8 weeks. Details of methods and analyses are given in Anderson *et al.* (1983).

At the end of the eighth week the animals were removed from the experimental materials and transferred to the litter or humus maintained under constantly moist conditions at the same experimental temperatures. The lids of the experimental chambers were not replaced so that the litter or humus could dry out. After 7 days the experimental materials were rewetted during the course of leachate sampling, as outlined above, the animals replaced and the chambers maintained in a moist condition for a further 7 days. The same procedure was followed for a further drying and wetting sequence.

3. Results

The time-course of ammonium-N and nitrate-N losses from litter at 15 °C are shown in Figure 1. The effects of the animals on nitrogen mineralization did not occur step-wise when the millipedes were added but developed to a maximum over a period of 3 weeks for ammonium-N and with a lag of a further week to reach maximum nitrate-N release. The animal treatments then maintained approximately twice the mineral nitrogen losses from the controls up to week 8. The desiccating atmosphere of the cooled incubators at 15 °C dries the materials in the chambers to constant mass, approximately 100 % moisture loss, in less than 3 days. These dry conditions supressed ammonium-N release while nitrate-N concentrations in leachates at the end of week 9 were less affected, though significantly reduced. By the end of week 10, after 7 days under the re-established moist regime, both ammonium-N and nitrate-N losses were significantly enhanced ($P < 0.05$) in relation to predrying concentrations; ammonium-N in controls by nearly 3 times the levels in week 8 (Fig. 1). Treatments with animals were significantly higher than controls after rewetting but the differences between treatments were less marked than during the continuously moist period up to week 8.



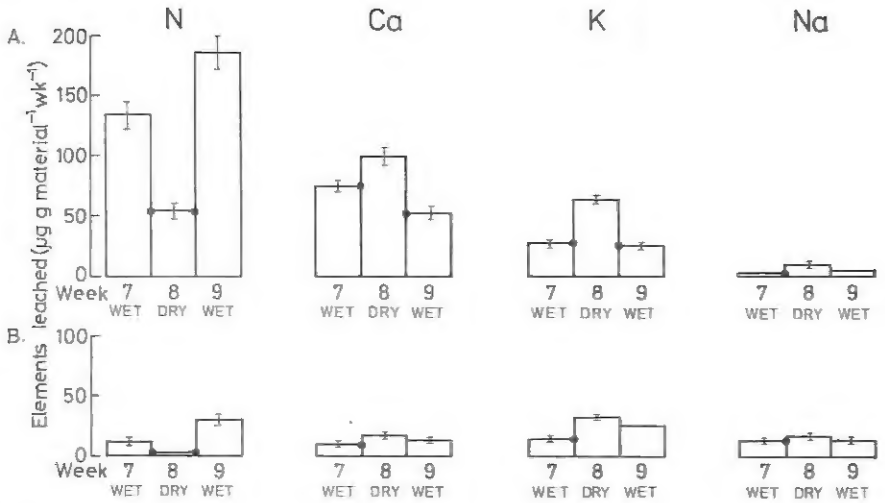


Fig. 2. Mineral-N, calcium, potassium and sodium losses (mean ± 1 SE) from leaf litter (A) and humus (B) materials in control treatments (without millipedes) in relation to a regime of drying and rewetting at 15 °C. The element concentrations were determined in distilled water used to leach the materials at the end of each period of seven days. Significant differences ($P < 0.05$) between element losses in consecutive weeks are indicated (●).

The pattern of nitrogen release was similar for the humus material at 15 °C, although mineral nitrogen losses were about 10 times smaller per gram resource (Fig. 2). The materials maintained at 10 °C and 5 °C showed progressively smaller responses than the treatments at 15 °C due in part to the less severe drying conditions in the cabinets. Materials at 10 °C were dry in 6 days while those maintained at 5 °C contained about 20% of the initial moisture content after 7 days.

The effects of the second drying regime (weeks 11 and 12) on nitrogen mineralization were closely similar to those for weeks 9 and 10 but the flush following rewetting was smaller and differences between treatments were not significantly different in most cases. Cumulative nitrogen losses from all treatments, for weeks 8 to 12, are shown in Table 1.

The losses of calcium, potassium and sodium from the litter and humus through wetting and drying showed a very different pattern to that for mineral nitrogen (Fig. 2). In all three cases the concentrations of the elements were significantly higher in leachates at the end of week 9, when the litter or humus was first rewetted, than after a further 7 days incubation under moist conditions. The pattern was similar in animal treatments though the element concentrations in leachates were significantly higher. As in the case of mineral nitrogen, the concentrations of elements released from humus were much smaller than those from litter and the animal effects were not generally significant (Table 1).

The release of calcium, potassium and sodium effected by the second drying was much smaller than for the first drying and element concentrations at the end of weeks 11 and 12, which were not significantly different in most cases, are not presented.

Fig. 1. Ammonium-N (A) and nitrate-N (B) losses from oak leaf (mean ± 1 SE) incubated at 15 °C for eight weeks and then subjected to alternate wet and dry periods of one week. Millipedes were removed from the animal treatments (●) before drying (see text) so that the traces during weeks 8 and 10 are shown in a similar manner to control treatments without animals (○).

Table 1. Cumulative losses (means ± 1 SE, n = 3) of mineral nitrogen, calcium, potassium and sodium from leaf litter and humus during the drying and rewetting regimes of weeks 8 to 12 carried out at different temperatures

Resource type	Element mobilized (μg g dry mass week $^{-1}$)	5 °C		10 °C		15 °C	
		Without animals	With animals	Without animals	With animals	Without animals	With animal
Litter (L/F)	Ammonium-N	134 ± 17	229 ± 38	148 ± 11*	246 ± 28	215 ± 11*	254 ± 23
	Nitrate-N	74 ± 9**	147 ± 14	111 ± 8**	172 ± 11	239 ± 15**	321 ± 16
	Total-N	208 ± 26***	375 ± 42	259 ± 10**	419 ± 37	455 ± 4**	575 ± 29
	Calcium	60 ± 8*	86 ± 15	92 ± 14**	161 ± 9	211 ± 25*	286 ± 9
	Potassium	134 ± 6*	166 ± 13	147 ± 4*	155 ± 6	153 ± 2	151 ± 2
	Sodium	18 ± 2**	30 ± 3	15 ± 1**	31 ± 1	14 ± 2***	38 ± 3
Humus (H)	Ammonium-N	48 ± 5	69 ± 10	22 ± 11*	47 ± 3	24 ± 1*	79 ± 20
	Nitrate-N	32 ± 0	27 ± 0	26 ± 10*	63 ± 4	35 ± 5**	120 ± 7
	Total-N	70 ± 4	96 ± 15	31 ± 5***	110 ± 7	63 ± 4**	199 ± 24
	Calcium	14 ± 3	13 ± 2	21 ± 2*	34 ± 6	51 ± 3**	85 ± 3
	Potassium	86 ± 5	77 ± 4	94 ± 10*	128 ± 5	130 ± 3	164 ± 9
	Sodium	46 ± 2	42 ± 2	38 ± 1*	53 ± 4	40 ± 2	50 ± 4

Details of treatments with and without millipedes are given in the text. Significant differences between treatments are shown as* ($0.001 < P \leqslant 0.01$) ** ($0.001 < P \leqslant 0.01$) and *** ($P \leqslant 0.001$).

4. Discussion

The temperature and moisture interactions in these experiment complicate interpretation since at 15 °C the litter and humus were subject to more severe desiccating conditions than at 5 °C. In addition, the flush of microbial activity following rewetting will show a faster response at higher temperatures. It was not considered desirable to independently vary the moisture content of the experimental materials since drying the litter or humus at low temperatures would have involved treatments, or different periods of time for air-drying, which would further complicate interpretation. These experiments were, however, carried out under conditions which were analogous to effects of dry periods during cool winter/spring or warmer summer/autumn soil conditions in our woodland field sites Anderson *et al.* (1985). The key issue is the suggestion by Witkamp & Frank (1970) that animal effects on nutrient mobilization are less significant than mobilization through wetting and drying at soil temperatures between 15 °C and 25 °C.

This discussion mainly concerns nitrogen mineralization since the effects of macrofauna on nitrogen fluxes are much larger than for calcium, potassium and sodium (Anderson *et al.* 1983a, b). The mobility of these cations is largely determined by abiotic leaching processes and the controls by fauna are small in relation to the total flux of these elements. Calcium is a partial exception since it requires biological mineralization processes to release the element from cell walls, whereas potassium and sodium are predominantly present as ions. But the general pattern of losses for all three elements is one of rapid initial leaching with the loss rates decreasing as the element concentrations approach those retained in microbial tissues (Gosz *et al.* 1973, Swift *et al.* 1979). Wetting and drying will mobilize these elements from microbial pools (Jager & Bruins 1976, Jenkinson & Ladd 1981) but, unlike nitrogen, a sequence of microbial mineralization processes is not required for the release of simple cations. This is shown in Figure 2 where the brief period of rewetting, of only a few minutes, associated with leaching the experimental chambers at the end of week 9, resulted in a significantly larger mobilization of calcium, potassium and sodium than at the end of week 10. By contrast, the release of mineral nitrogen through ammonification and nitrification involves the recovery of the microflora after rewetting and the flush of nitrogen resulting from drying occurred at the end of week 10 (Fig. 2).

The much smaller flush of mineral nitrogen from the humus dried at 15 °C, than from the litter, is unrelated to the nitrogen concentrations of the two resource types (1.27 % for litter and 1.53 % for humus) and reflects the reduced availability of nitrogen to microorganisms with the advancing stages of decomposition (Hayes & Swift 1978, Sollins *et al.* 1984). However, while these differences are reflected in the cumulative total of mineral nitrogen mobilized from litter and humus at the different temperatures (Table 1), the proportional effects of the millipedes in relation to the controls are different. In the litter material the proportionally larger animal effects are manifest at 5 °C, where total mineral nitrogen released from animal treatments are 80 % higher than controls. At 15 °C animal treatments are only 26 % higher than controls as a consequence of the dry periods inhibiting the maintenance of the millipede/microbial interactions which take several weeks to develop. The effects of the dry periods are, in effect, similar to removing the animals completely (Anderson & Ineson 1984).

In the humus, however, the effects of the animals were nonsignificant at 5 °C but at 10 °C and 15 °C the animal treatments lost more than twice the total mineral nitrogen than was mobilized from controls.

These results do not support in detail the conclusions of Witkamp & Frank (1970). Firstly, the effects of animal feeding activities or wetting and drying on the release of simple cations from litter and soil organic matter are generally small in relation to the total fluxes of the elements. Secondly, although the drying inhibits the ongoing effects of the animals on nitrogen mineralization, materials which have been subject to animal feeding, and particularly the humus, have a larger pool of labile nitrogen than the controls subject to microbial activity alone.

The magnitude of the wetting and drying effects on nitrogen mineralization at high temperatures therefore depends upon the nature and activities of the whole soil biota for several weeks before drying occurs. We are able, in part, to substantiate these effects under field conditions. Small lysimeters (0.5 m × 0.5 m) have been set up in an oak woodland to determine animal effects on nitrogen mineralization at the ecosystem scale (ANDERSON *et al.* 1985). Treatments with macrofauna (14—20 g fresh mass m^{-2}) and controls from which macrofauna are excluded (micro- and meso-fauna populations are assumed to be similar in all treatments) were established in April 1983. During July and August 1983 there were three periods with no rainfall when mean weekly soil temperatures were between 15 °C and 18 °C and the litter and humus became very dry. On rewetting by isolated storms up to 3.1 kg ha^{-1} ammonium-N and 1.27 kg ha^{-1} nitrate-N were released from animal treatments during the following week in comparison with 1.93 kg ha^{-1} ammonium-N and 1.30 kg ha^{-1} nitrate-N from the controls; a difference of 3.8 kg ha^{-1} total mineral nitrogen from the animal treatments.

These experiments are of a preliminary nature but demonstrate the need for more understanding of the mechanisms of nitrogen mineralization in forest soils. Specifically information is needed on the intensity, frequency and duration of dry periods in influencing the location of macrofauna in the soil and litter horizons, and the mobilization of labile nitrogen pools formed through their feeding activities.

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Leaf litter and humus materials from a predominantly oak woodland were incubated at 5 °C, 10 °C and 15 °C with and without millipedes for eight weeks. The animal feeding activities substantially increased mineral nitrogen (as nitrate and ammonium) and calcium losses from the experimental materials while the effects on potassium were less marked. The animals were then removed while the samples were subjected to a drying regime for a week and replaced when the materials were rewetted. At 15 °C both litter and humus samples were desiccated within 7 days while at 10 °C and 5 °C drying was incomplete. Drying and rewetting enhanced mineral losses, particularly mineral-N, though the effects on K and Na were smaller and non-significant in several cases. The flush of Ca, K and Na immediately followed rewetting of the litter and humus while the flush of mineral-N occurred over the following 7 days. Drying and rewetting enhances the mobilization of a larger labile N pool in animal treatments than the controls. But the experimental regime of alternating weeks of wet and dry conditions does not allow the time necessary for the animal enhancement of nitrogen mineralization to develop and is similar in effect to removing the animals completely.
Key words: Animal/microbial interactions, nitrogen mineralization, drying, millipedes, deciduous woodland, litter, humus.